APPLICATION FOR PATENT

Title: NOVEL CHEMOKINE BINDING PEPTIDES CAPABLE OF MODULATING THE BIOLOGICAL ACTIVITY OF CHEMOKINES

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This Application is a Continuation-in-Part of PCT Application No. PCT/IL03/00155, filed 27-February-2003, currently pending, which claims priority from 60/359,995, filed 28-Feb-2002, now expired, all of which are hereby incorporated by reference as if fully set forth herein.

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FIELD OF THE INVENTION

The present invention discloses novel peptidic molecules or peptidomimetic agents, which are capable of binding chemokines and modulating their biological functions.

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BACKGROUND OF THE INVENTION

Drug discovery in the post-genomics era provides enormous opportunities as well as new challenges. The targets of the drug discovery process have changed greatly over the last 50 years. The development of

20   advanced purification technologies and the tools of molecular biology have brought molecular targets into the current discovery process. In the last ten years, there has been a trend towards selecting molecular targets for the screening process, and the human and other genome projects have made available many thousands of additional targets for drug discovery.

25   In addition to these novel targets with unknown potential, there are a significant number of well-validated targets associated with major human diseases. Most of these are either nuclear receptors or G protein-coupled receptors. It was found, that in some cases, one compound that has an effect through one receptor, can also act through another receptor, and that several

compounds can work through the same receptor. Unfortunately, even when the mechanism of a disease process is understood, for example by identifying the receptor(s) responsible for such a process, this information has not always resulted in the development of new treatments. For example, subjects who

5 suffer from inflammation associated diseases and disorders have a great and desperate demand for novel drugs as therapeutic agents. Current therapies are merely palliative and have not been significantly improved in recent years.

Recent scientific advances provide some hope that new treatments will soon be available for these diseases. Sequencing of the human genome, which

10 contains nearly 30,000 genes, has been recently completed. This significant achievement in the frontiers of human medicine will allow the identification of genes involved in the onset and progression of human diseases and pathological states. Many of these genes will serve as valid targets in the discovery process of drugs that are more effective in the treatment of inflammatory diseases.

15 Along with a massive flow of novel genes with potential therapeutic properties, there is a growing need for more rapid and efficient ways to discover lead compounds with enhanced (agonistic) or inhibitory (antagonistic) properties.

Chemokines are among the biological factors that are, amongst other functions, involved in the inflammatory disease process. Chemokines belong

20 to a group of small, ~8-14 kDa, mostly basic, heparin binding proteins that are related both in their primary structure and the presence of 4 conserved cysteine residues. The chemokines are chemotactic cytokines that have been shown to be selective chemoattractants for leukocyte sub-populations *in vitro*, and to

elicit the accumulation of inflammatory cells *in vivo*. In addition to chemotaxis, chemokines mediate leukocyte de-granulation (Baggiolini and Dahinden, 1994) and the up-regulation of adhesion receptors (Vaddi and Newton, 1994), and have recently been implicated in the suppression of human immunodeficiency virus replication (Cocchi et al., 1995).

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Chemokines can be divided into 4 groups (CXC, CX3C, CC, and C) according to the positioning of the first 2 closely paired and highly conserved cysteines of the amino acid sequence. The specific effects of chemokines on their target cells, are mediated by members of a family of 7-transmembrane-spanning G-protein-coupled receptors. These chemokine receptors are part of a much bigger super family of G-protein-coupled receptors that include receptors for hormones, neurotransmitters, paracrine substances, inflammatory mediators, certain proteinases, taste and odorant molecules and even photons and calcium ions.

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The chemokine receptors have received increasing attention due to their critical role in the progression of immune disease states such as asthma, atherosclerosis, graft rejection, AIDS, multiple sclerosis and others. It would be useful to have therapeutic agents capable of inhibiting the binding of ligands of some chemokine receptors in order to lessen the intensity of or cure these diseases. Chemokines themselves play an essential role in the recruitment and activation of cells from the immune system. They also have a wide range of effects in many different cell types beyond the immune system, including for example, in various cells of the central nervous system (Ma et al., 1998) or

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endothelial cells, where they result in either angiogenic or angiostatic effects (Strieter et al., 1995). Recent work has shown that particular chemokines may have multiple effects on tumors, including promoting growth, angiogenesis, metastasis, and suppression of the immune response to cancer, while other chemokines inhibit tumor mediated angiogenesis and promote anti-tumor immune responses.

Recently, it was shown that the SDF-1α/CXCR4 chemokine/chemokine receptor pathway is involved in dissemination of metastatic breast carcinomas (Muller A, 2001). This example illustrates that both chemokines and their receptors are potentially valuable targets for therapeutic intervention in a wide range of diseases.

SUMMARY OF THE INVENTION

The background art does not teach or suggest sequences or compositions containing peptidic modulators capable of binding to chemokines and inhibiting or activating their biological functions. The background art also does not teach or suggest sequences or compositions containing the basic consensus sequences that characterize families of such peptidic chemokine-binding modulators. In addition, the background art does not teach or suggest the nucleic acid molecules encoding for such peptidic chemokine-binding modulators. Finally, the background art does not teach or suggest methods of treatment employing such peptidic chemokine-binding modulators.

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The present invention overcomes these deficiencies of the background art by providing peptidic chemokine-binding modulators, with defined amino acid sequences, which have been found to bind to specific chemokines, including but not limited to human SDF-1α, MIG, IL-8, MCP-1 and Eotaxin,

5   and which modulate the binding of these chemokines to their respective receptors and/or which otherwise have an inhibitory or stimulatory effect on the biological activity of chemokines.

It should be noted that the term "peptidic" as used herein, also includes peptidomimetics and hybrid structures comprised of at least one amino acid and

10   at least one molecule that is not an amino acid, or at least not a naturally occurring amino acid. The term amino acid may optionally also include non-naturally occurring amino acids as well as naturally occurring amino acids, and derivatives and analogs thereof.

A peptide mimetic (peptidomimetic) is a molecule that mimics the

15   biological activity of a peptide, yet is no longer peptidic in chemical nature. By strict definition, a peptidomimetic is a molecule that no longer contains any peptide bonds, i.e., amide bonds between amino acids; however, in the context of the present invention, the term peptide mimetic and also the term peptidomimetic are intended to include molecules that are no longer completely

20   peptidic in nature, such as pseudo-peptides, semi-peptides and peptoids. Whether completely or partially nonpeptide, peptidomimetics according to the present invention provide a spatial arrangement of reactive chemical moieties that closely resembles the three-dimensional arrangement of active groups in

the peptide on which the peptidomimetic is based. The techniques of developing peptidomimetics are conventional. Thus, non-peptide bonds that allow the peptidomimetic to adopt a similar structure to the original peptide can replace peptide bonds.

5   Replacing chemical groups of the amino acids with other chemical groups of similar structure can also be used to develop peptidomimetics.

Preferably, the present invention features basic consensus sequences, some of which characterize families of such peptidic chemokine-binding modulators.

10   More specifically, if the biological activity caused by the target protein, i.e. chemokine receptor, when activated through binding the target ligand, i.e. chemokine, involves activation of some biological function, then the inhibitory peptide preferably inhibits such activation. On the other hand, if the biological activity caused by the receptor involves inhibition of some biological function,

15   then the inhibitory peptide preferably blocks inhibition of the biological function.

According to another preferred embodiment of the present invention, there is provided a composition for treating inflammatory and cancer metastasis conditions in a subject, comprising a pharmaceutically effective amount of a

20   therapeutic agent for administering to the subject, the therapeutic agent being composed of the peptidic chemokine-binding modulators as the active ingredient as well as a suitable pharmaceutical carrier if necessary.

According to a preferred embodiment of the present invention, the therapeutic agent may be administered topically, intranasally and by inhalation. Alternately, the therapeutic agent may be administered by systemic administration.

5   According to the present invention, there is provided a peptidic chemokine modulator for modulating a biological effect of a chemokine, comprising a molecule having a defined amino acid composition.

According to another embodiment of the present invention, there is provided a peptidic chemokine modulator for modulating a biological effect of

10   a chemokine, comprising a molecule composed of the amino acids H, S, A, L, I, K, R, T and P, and featuring at least 2 Histidines spread along the molecule, wherein the molecule features an overall positive charge (family 1). The present invention comprises peptides having these characteristics and preferably being up to about 20 amino acids in length, more preferably from

15   about 10 to about 20 amino acids in length, and optionally and most preferably about 12 amino acids in length. Preferably, the molecule comprises a peptide having an amino acid sequence selected from the group consisting of SIFAHQTPTHKN (seq id no:100), SIPSHSIHSAKA (seq id no:101), SAISDHRAHRSH (seq id no:96), SAGHIHEAHRPL (seq id no:95),

20   ACHASLKHRC (seq id no. 44), AHSLKSITNHGL (seq id no:46), ESDLTHALHWLG (seq id no:54), HSACHASLKHRC (seq id no:69), WSAHIVPYSHKP (seq id no:143), YATQHNWRLKHE (seq id no:145), CAHLSPHKC (seq id no:1), GVHKHFYSRWLG (seq id no:61),

7

HPTTPFIHMPNF (seq id no:66), SVQTRPLFHSHF (seq id no:113), and

VHTSLLQKHPLP (seq id no:133). More preferably, the peptide has an amino

acid sequence SIFAHQTPTHKN (seq id no:100). The peptidic chemokine

modulator may optionally be used for binding to a chemokine selected from the

5   group comprising MIG, MCP-1, IL-8, SDF-1 alpha and Eotaxin.

According to another embodiment of the present invention, there is

provided a peptidic chemokine modulator for modulating a biological effect of

a chemokine, comprising a molecule composed of the amino acids H, P, T, L,

R, W, F, and featuring at least two neighboring histidines, wherein the

10   molecule features an overall positive charge (family 2). The present invention

comprises peptides having these characteristics and preferably being up to

about 20 amino acids in length, more preferably from about 10 to about 20

amino acids in length, and optionally and most preferably about 12 amino acids

in length. Preferably, the molecule comprises a peptide having an amino acid

15   sequence selected from the group consisting of GDFNSGHHTTTR (seq id

no:59), HHFHLPKLRPPV (seq id no:64), HHTWDTRIWQAF (seq id no:65),

LDYPIPQTVLHH (seq id no:76), LLADTTHHRPWP (seq id no:79),

TRLVPSRYYHHP (seq id no:125), CHHNLSWEC (seq id no:7) and

SFWHHHSPRSPL (seq id no:99). More preferably, the peptide has an amino

20   acid sequence LLADTTHHRPWP (seq id no 79:).

According to another embodiment of the present invention, there is

provided at least one peptide having at least 90% sequence homology, more

preferably about 95% sequence homology, and most preferably sequence

8

identity to any peptide listed in any of the Tables herein and/or in the specification, that is preferably up to about 20 amino acids in length, more preferably from about 10 to about 20 amino acids in length, and optionally and most preferably about 12 amino acids in length. A peptide according to the

5   present invention may optionally belong to one of the two families, or may alternatively not belong to either family.

Hereinafter, homology is defined as that which is determined with the Smith-Waterman algorithm, using the Bioaccelerator platform developed by Compugene (gapop: 10.0, gapext: 0.5, matrix: blosum62).

10   According to another embodiment of the present invention, there is provided a composition for treating a condition involving abnormal cell migration in a subject, the composition comprising a pharmaceutically effective amount of a therapeutic agent for administering to the subject, the therapeutic agent comprising a chemokine modulator as described above. Preferably, the

15   condition comprises an inflammatory condition. Alternatively, the condition comprises cancer metastasis. Optionally, the therapeutic agent is administered by topical administration, such that the composition further comprises a pharmaceutically acceptable carrier for topical administration. Preferably, the topical administration is to the skin of the subject. Optionally, the therapeutic

20   agent is administered by inhalation, such that the composition further comprises a pharmaceutically acceptable carrier for inhalation. Alternatively, the therapeutic agent is administered intranasally, such that the composition further comprises a pharmaceutically acceptable carrier for intranasal

administration.

Optionally, the therapeutic agent is characterized by an ability to inhibit binding of the chemokine to a chemokine receptor.

Optionally, the therapeutic agent is characterized by an ability to enhance binding of the chemokine to a chemokine receptor.

According to another embodiment of the present invention, there is provided a method for treating a disease modulated through and/or caused by binding of a chemokine to a chemokine receptor in a subject, comprising administering a pharmaceutically effective amount of a therapeutic agent to the subject, the therapeutic agent comprising a peptidic chemokine modulator as described above.

Preferably, the therapeutic agent binds to at least one of the chemokines and wherein the therapeutic agent directly modulates the activity of the chemokine by modulation of binding to the chemokine receptor.

Optionally and preferably, the disease is selected from the group consisting of: inflammation (primary or secondary), allergy, a non-optimal immune response, an autoimmune reaction (including rheumatoid arthritis, systemic lupus erythematosis, multiple sclerosis and others), allograft rejection, diabetes, sepsis, cancer and any type of malignant cell growth, acute and chronic bacterial and viral infections, arthritis, colitis, psoriasis, atherosclerosis, hypertension and reperfusion ischemia.

According to still another embodiment of the present invention, there is provided an antibody for binding to a chemokine-binding receptor, comprising:

an antibody being capable of recognizing at least a portion of a chemokine-binding receptor, wherein the antibody also recognizes a peptide having a sequence as described above.

Optionally, there is also provided a vaccine formed with the above antibody.

According to still another embodiment of the present invention, there is provided a method for producing an antibody, comprising: inducing formation of antibody against a peptide having a sequence according to the above description, wherein the antibody is also capable of recognizing a chemokine-binding receptor.

Preferably, the antibody comprises a monoclonal antibody. Alternatively, the antibody comprises a polyclonal antibody. Preferably, the antibody forms a vaccine.

The peptidic chemokine-binding modulators are then preferably used to develop one or more lead compounds for new therapies. Alternatively or additionally, the peptidic chemokine-binding modulators themselves may have therapeutic value, and as such, may optionally be used for treatment of a subject.

Also additionally or alternatively, binding of the peptidic chemokine-binding modulators may optionally be used to identify lead proteins, which are reference proteins whose reactivity descriptors are substantially similar to those of the protein of interest such as novel chemokine binding proteins. By "reactivity descriptor" it is meant the characteristics of binding to other

chemokines and/or chemokine binding proteins, and/or the biological activity induced and/or inhibited by the reference protein.

Also additionally or alternatively, the peptidic chemokine-binding modulators may optionally be used as antigens to produce antibodies to these

5   peptides, which can also bind chemokine-binding receptors, or optionally may be used to stimulate the production of auto-antibodies against these peptides that can also bind chemokine receptors. The latter use would involve using these peptides (or other modulators) as a vaccine, optionally with any suitable vaccine carrier that could easily be selected by one of ordinary skill in the art,

10   including but not limited to, adjuvants, carriers and the like. More preferably, the modulators that are used to produce the antibodies are selected from one of the peptides described herein. It should be noted that the vaccine may also optionally comprise the antibody itself.

Hereinafter, the term "biologically active" refers to molecules, or

15   complexes thereof, which are capable of exerting an effect in a biological system. Hereinafter, the term "fragment" refers to a portion of a molecule or a complex thereof, in which the portion includes substantially less than the entirety of the molecule or the complex thereof.

Hereinafter, the term "amino acid" refers to both natural and synthetic

20   molecules that are capable of forming a peptide bond with another such molecule. Hereinafter, the term "natural amino acid" refers to all naturally occurring amino acids, including both regular and non-regular natural amino acids. Hereinafter, the term "regular natural amino acid" refers to those alpha

12

amino acids that are normally used as components of a protein. Hereinafter, the term "non-regular natural amino acid" refers to naturally occurring amino acids, produced by mammalian or non-mammalian eukaryotes, or by prokaryotes, which are not usually used as a component of a protein by

5   eukaryotes or prokaryotes. Hereinafter, the term "synthetic amino acid" refers to all molecules which are artificially produced and which do not occur naturally in eukaryotes or prokaryotes, but which fulfill the required characteristics of an amino acid as defined above. Hereinafter, the term "peptide" includes both a chain and a sequence of amino acids, whether

10   natural, synthetic or recombinant. Hereinafter, the term "peptidomimetic" includes both peptide analogues and mimetics having substantially similar or identical functionality thereof, including analogues having synthetic and natural amino acids, wherein the peptide bonds may be replaced by other covalent linkages.

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BRIEF DESCRIPTION OF THE DRAWINGS AND TABLES

The invention is herein described, by way of example only, with reference to the accompanying drawings and tables, wherein:

FIG. 1 shows the results of binding of chemokines to BKT-P1, BKT-P3,

20   BKT-P22, and BKT-P37 peptides, which bind, respectively, to at least one of the following chemokines, SDF-1α, MCP-1, Eotaxin and IL-8;

FIG. 2 shows the binding of synthetic peptides from Table 1 which can either bind specifically to various chemokines (such as BKT-P1, shown in Fig.

2B), or can alternatively bind specifically to a single chemokine (such as BKT-P9 alone, as shown in Fig 2A);

FIG. 3 shows the results of binding of one of the synthetic peptides from family 1, BKT P3, to various chemokines. BKT-P18, which does not belong to the family, is shown as a control;

FIG. 4 shows a graphical representation of the biological activity results for BKT-P3, from family 1 in a biovalidation assay system in which the chemokine MIG and the adhesion receptor VCAM-1 are used to activate the binding of T cells (see "Material and Methods") (FIG. 4A); BKT-P10, which does not belong to the family, is shown as a control (FIG. 4B).

Fig. 5 shows a graphical representation of the level of immunity (O.D.) of antibody raised against BKT-P3 and BKT-P2, both of which belong to family 1, to the relevant peptides, BKT-P3 and BKT-P2. The preimmune serum of the same mice used as a control. "Blank" is the O.D. level of antibody binding to the plastic (of the Elisa experiment), with no addition of peptides.

Table 1 shows the sequences of peptides that bind to the chemokines MCP-1, SDF-1α, MIG, Eotaxin and IL-8;

Table 2 shows a family of peptides (family no. 1) that bind to MIG, MCP-1, IL-8, SDF-1α and Eotaxin and are predominantly composed of the amino acids H, S, A, L, I, K, R, T and P, featuring at least 2 Histidines spread along the molecule. The abundance of positively charged amino acids such as H, K and R, resulted in peptides having an overall positive charge. The remaining

amino acids, mentioned above, might participate in the determination of the three dimensional structure of the peptides;

Table 3 shows a family of peptides (family no. 2) that bind mostly to MCP-1 and in individual cases to IL-8, SDF-1α and Eotaxin; the binding motif for peptides in this family is predominantly composed of the amino acids H, P, T, L, R, W, F, featuring at least 2 Histidines next to each other. The abundance of positively charged amino acids, such as H and R, resulted in peptides having an overall positive charge. The remaining amino acids, mentioned above, might participate in the determination of the three dimensional structure of the peptides;

Table 4 shows a summary of the results of the biological activity of four representatives from family 1, in a biovalidation assay system, in which various chemokines and the adhesion receptor VCAM-1 are used to activate the binding of T cells (as described in "Materials and Methods").

Table 5 shows a summary of the results of the biological activity of two representatives from Table 1, which do not belong to either family 1 or family 2, in a biovalidation assay system, in which various chemokines and the adhesion receptor VCAM-1 are used to activate the binding of T cells (as described in "Materials and Methods").

Table 6 shows the list of synthetic peptides that were ordered for further analysis of their ability to modulate chemokine activity.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

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The present invention is composed of peptidic modulatory molecules with defined amino acid sequences which have been found to bind to specific chemokines, including, but not limited to, human SDF-1α, MIG, IL-8, MCP-1 and Eotaxin, and which inhibit or stimulate the binding of these chemokines to

5   their respective receptor/s and/or which otherwise have an inhibitory or stimulatory effect on the biological activity of chemokines. Preferably, the present invention specifies basic consensus sequences, with overall positive electrostatic charge, which characterize families of such modulatory chemokine-binding peptide molecules.

10   The chemokine binding peptides mimic chemokine receptor structures or motifs. It is therefore predicted that the chemokine-binding peptides, when injected or otherwise introduced into mice or humans, may produce antibodies against chemokine receptors that can be either used for the production of monoclonal antibodies or used for development of a vaccine against a

15   particular chemokine receptor/s. Whether antibodies are produced, or alternatively, binding to a particular chemokine receptor is blocked and/or enhanced, may depend upon the method of introduction to the subject. For example, antibody production may be potentiated if the peptides (and/or other peptidic modulatory molecule according to the present invention) are

20   introduced with an adjuvant. Concentration of the peptide or other molecule may also be important. In any case, one of ordinary skill in the art could easily determine conditions for antibody production, as opposed to having the effect

16

potentiated or mediated directly through the peptides and/or other peptidic modulatory molecules according to the present invention.

The modulatory chemokine-binding peptide molecules of the present invention could therefore be useful for treating a disease selected from the group consisting of inflammation (primary or secondary e.g. uveitis, bowel inflammation), allergy, non-optimal immune response, autoimmune reaction (including rheumatoid arthritis, systemic lupus erythematosis, multiple sclerosis and others), delayed-type hypersensitivity, allograft rejection, diabetes, sepsis, cancer and any type of malignant cell growth, (including, but not limited to breast cancers such as infiltrating duct carcinoma of the breast or other metastatic breast cancers, lung cancers such as small cell lung carcinoma, bone cancers, bladder cancers such as bladder carcinoma, rhabdomyosarcoma, angiosarcoma, adenocarcinoma of the colon, prostate or pancreas, or other metastatic prostate or colon cancers, squamous cell carcinoma of the cervix, ovarian cancer, malignant fibrous histiocytoma, skin cancers such as malignant melanoma, lymphomas and leukemia, leiomyosarcoma, astrocytoma, glioma and heptocellular carcinoma), acute and chronic bacterial and viral infections, vasculitis, arthritis, colitis, psoriasis, atherosclerosis, Graves disease, anorexia nervosa; hemorrhagic shock caused by septicemia, HIV infection in AIDS, pemphigus, asthma, renal diseases, liver diseases, bone marrow failure, vitiligo, alopecia, and myositis, hypertension and reperfusion ischemia.

The examples provided below, describe certain experiments performed with the peptidic modulatory of the present invention, demonstrating the

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binding efficacy of these molecules to the various chemokines. It also demonstrates the efficacy of the peptidic chemokine-binding modulator in a biological system. In addition, the examples also describe formulations for administering the compounds of the present invention, and methods of

5   treatment thereof.

EXAMPLE 1

EFFICACY OF THE PEPTIDES OF THE PRESENT INVENTION

This Example demonstrates the efficacy of the peptidic chemokine-

10   binding modulators of the present invention, in a number of different assays, including binding assays and assays for measuring a functional biological effect.

Materials and methods

15   **Chemokines**

Recombinant chemokines were ordered from PeproTech, Inc. (Rocky Hill, NJ, USA). Human SDF-1α (Cat. No. 300-28A), human MIG (Cat. No. 300-26) and human IL-8, (72 amino acids) (Cat. No. 200-08M), belong to the alpha-chemokines (C-X-C) family. Human MCP-1 (MCAF) (Cat. No. 300-04)

20   and human Eotaxin (Cat No. 300-21) belong to the beta-chemokines (C-C family). All chemokines were prepared according to the company recommendations.

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Peptide synthesis

Peptides were synthesized in the Weizmann Institute of Science, Rehovot, Israel, in order to perform tests for characterization of their influence on the biological activity of the chemokines. The format of the various

5   synthesized peptides was as follows: The cyclic peptides, $ACX_7CGGGSK$-biotin-G and the linear peptides, $X_{12}GGGSK$-biotin-G. The peptides were biotinylated on their C-termini; the biotin will serve as a detector during the following experiments. Each synthetic peptide was dissolved to concentration of 1mg/ml (~0.6 mM) in 4% DMSO (Dimethyl Sulphoxide, Sigma, Cat. # D-

10   2650).

ELISA analysis of the synthetic chemokine-binding peptide

NUNC-Immuno maxisorp plates (Cat. No. 4-42404) were coated with the appropriate chemokine (0.1 ml/well, 0.1-1.0 µg/ml in 0.1 M $NaHCO_3$, pH

15   8.6), overnight at 4^0C. The plates were then blocked with 0.2 ml/well of blocking buffer (5mg/ml BSA in 0.1 $NaHCO_3$). Control wells were treated with blocking buffer alone, with no addition of target protein (chemokine). The plates were washed 6 times with PBST (0.1% Tween 20 in PBS), followed by incubation for 45 minutes at room temperature with 10-fold serial dilutions of

20   individual synthetic peptides (10pg-10µg) with 1%BSA (PBST-BSA)/well. After the plates were washed 6 times with PBST, the bound peptides were probed by HRP-SA Conjugate, diluted 1:10,000 to 1:20,000 in PBST-BSA, 0.1 ml/well for 45 minutes at room temperature. The target-bound synthetic

peptides probed with HRP-SA were quantified by DAKO TMB one–step substrate system, followed by the addition of stop solution, $HCl-H_2SO_4$ mixture (0.1 ml/well) (1N HCl, 3N H_2SO_4). The results were analyzed by ELISA reader at OD_{450}.

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T-Cell purification from fresh blood

50ml blood was added to 10ml Dextran (Dextran T-500 6% w/v) in PBS (phosphate buffer saline), and 7ml citrate buffer (25g citrate, 8g citric acid in 500ml PBS). The solution was incubated for 30 min at 25° C. 10ml Ficoll 1077

10 (Sigma) was added to the bottom of the tube. The tube was then centrifuged at 2,000 rpm for 30 min, at 18° C, (with the brake mode of the centrifuge off). The interphase was collected and washed twice with 8ml PBS-5% FCS (fetal calf serum), followed by centrifugation at 1,400 rpm, for 5 min, at 18^OC. The cells were re-suspended in PBS-5% FCS at a concentration less then 10^8/ml.

15 2ml of the cell solution were applied and incubated for 45 min at 25° C on a Perspex Nylon wool column, which was pre-soaked with PBS-5% FCS. Each column was washed with 8ml PBS-5% FCS and the cells (T-cells and erythrocytes) were eluted by 50ml of 5mM EDTA in PBS. A red pellet was obtained by centrifugation at 1,400rpm, at 4^OC, for 5min, with the brake on. In

20 order to perform lysis of the erythrocytes, the red pellet was re-suspended in 5ml lysis-buffer (155mM NH_4Cl, 10mM $KHCO_3$, 0.1mM EDTA, X0.1PBS) for 4 min, followed by immediate addition of 50ml of PBS-EDTA.

Following centrifugation at 1,400rpm, at 4°C, for 5min with brake on, the pellet was washed again with 50ml PBS-EDTA and re-centrifuged under the same conditions. A white pellet was obtained and re-suspended in RPMI/10% FCS/L-glutamine/sodium pyruvate/antibiotics at a concentration of $3^{X}10^{6}$cells/ml. The cells were incubated for 2h at 37^{0}C, followed by collection of the non-adherent cells. The cells were ready for use in experiments after overnight incubation at 37^{0}C.

Preparation of adhesive substrates

Human VCAM-1 (1μg/ml) and SDF-1α (intact or heat-inactivated) (2μg/ml) were dissolved in PBS buffered with 20mM bicarbonate, pH 8.5, and incubated on a polystyrene plates overnight at 4 °C. The plates were then washed three times and blocked with human serum albumin (20mg/ml in PBS) for 2 h at 37°C.

Biovalidation

Laminar flow assays were performed as follows. Polystyrene plates (B.D) were coated with soluble VCAM-1 at 10 μg/ml in the presence of 2 μg/ml HSA carrier. The plates were washed three times with PBS and blocked with HSA (20 μg/ml in PBS) for 2 hrs at room temperature. Alternatively, washed plates were coated with 10 μg/ml MIG chemokine in PBS for 30 min at room temperature, before being blocked with HSA. The plates were assembled as the lower wall of a parallel wall flow chamber and mounted on the stage of

21

an inverted microscope. The peptide, as described previously, (10μg/ml) was allowed to settle on the substrate coated chamber wall for 10 min, at 37^{0}C and then washed. T cells (5x10^6/ml, purity >98%) were suspended in binding buffer, perfused into the chamber and allowed to settle on the substrate coated

5   chamber wall for 1 min, at 37^{0}C. Flow was initiated and increased in 2 to 2.5 fold increments every 5 sec. generating controlled shear stresses on the wall. Cells were visualized in a 20x objective of an inverted phase-contrast Diaphot Microscope (Nikon, Japan) and photographed with a long integration LIS-700 CCD video camera (Applitech; Holon, Israel), connected to a video recorder

10   (AG-6730 S-VHS, Panasonic, Japan). The number of adherent cells resisting detachment by the elevated shear forces was determined after each interval by analysis of videotaped cell images, and was expressed as the percent of originally settled cells. All adhesion experiments were performed at least three times on multiple test fields.

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Results

Identification of chemokine binding peptides with antagonistic effect

The present invention identifies chemokine-binding peptides with biological activity. The binding specificities of the various peptides to the

20   chemokines were determined by screening against BSA, Actin and Fibronectin-coated wells as negative controls in parallel experiments. The binding level of the peptides to the various chemokines, which reached at least two fold of the

binding level to the control proteins, was considered a specific binding. The different peptides that were found to bind to chemokines, are listed in Table 1.

Graphical representations of four examples of such peptide-containing carriers, from the list in Table 1, are shown in Figure 1. As can be seen, each of those peptides was found to bind to at least one different chemokine, which was chosen to be presented in this graph: peptide BKT-P1 was found to bind SDF-1α; peptide BKT-P3 was found to bind, in this representation, to MCP-1; peptide BKT-P22, was found to bind Eotaxin and peptide BKT-P37, was found to bind IL-8. The specificity of the binding was calculated by comparing the binding level of the proteins to chemokines to that of binding level to control proteins, as explained above. It should also be noted that the binding of each of the peptides shown in Figure 1 is not necessarily the only binding capability shown by those peptides.

Peptides that showed affinity/binding to one or more chemokines were then analyzed in several individual experiments, and were chosen for further analysis. The specific binding of the peptides was detected by screening methodology, using ELISA, as described in "Materials and Methods", employing microplates coated with the various chemokines to be checked. The binding specificities of the various peptides to the various chemokines were determined by screening against BSA, Actin and Fibronectin-coated wells as negative controls in parallel experiments.

As can be seen in Figure 2, peptides that can specifically bind to one specific chemokine, such as BKT-P9, were identified (Fig. 2A). In addition,

23

peptides that can bind, specifically to more than one chemokine, such as BKT-P1, were also identified (Fig. 2B). Again, the specificity of the binding, either to one chemokine or more, was established by comparing binding level with the control binding level to non-related proteins, (shown in the Figure as a broken line). Control binding showed a level of one fold increase. As such, each of the peptides that showed a level of binding to a particular chemokine of less than, or close to, such a one-fold increase, was considered to be non-specifically bound to that chemokine. Specific binding was considered as that which showed at least a two fold increase over the control binding level. Peptides that specifically bound to one or more chemokines were chosen for further examination and for analysis with a biovalidation assay, to prove their ability to not only bind the various chemokines but also to modulate the biological activity of those chemokines.

According to the present invention, two families of chemokine-binding peptides were identified. These families contain peptides with similar amino acid compositions, a high percentage of histidines, and are also characterized by overall positive electrostatic charge.

A detailed list of the potential consensus sequences of the two families is presented in Tables 2 and 3. The peptides of family no. 1 (Table 2) bind to MIG, MCP-1, IL-8, SDF-1α and Eotaxin, as illustrated in Table 2, and are predominantly composed of the amino acids H, S, A, L, I, K, R, T and P. Each peptide in this family contains at least 2 histidines distributed along the molecule. The abundance of positively charged amino acids such as H, K and

24

R, results in peptides having an overall positive charge. The remaining amino acids, mentioned above, might participate in the determination of the three dimensional structure of the peptides.

Table 3 shows a family of peptides (family no. 2) that bind mostly to

5 MCP-1 and in individual cases to IL-8, SDF-1α and Eotaxin (illustrated in the Table). The binding motif for peptides in this family is predominantly composed of the amino acids H, P, T, L, R, W, F, while each peptide also features at least two histidines, one next to the other. The abundance of positively charged amino acids such as H and R, resulted in peptides having an overall positive charge. The

10 remaining amino acids, mentioned above, may participate in the determination of the three dimensional structure of the peptides.

As defined here, a consensus sequence is composed of an amino acid sequence that is found repeatedly in a group of peptides that bind various chemokines and probably have certain biological functions in common. The

15 group or family of such peptides is characterized by the consensus sequence, high abundance of the amino acid histidine and overall positive electrostatic charge. These peptides may be described as potential agonists or antagonists of chemokines.

The binding of one synthetic peptide, BKT-P3 (family 1), to various

20 chemokines is shown in Figure 3. As can be seen, this peptide binds to all five chemokines tested, in contrast to the control peptide, BKT-P18, which does not belong to the family, and also shows no binding to the chemokines. As discussed above, the level of binding was calculated by comparing binding of

the peptides to the chemokines with peptide binding to controls. Level of control binding was defined as level 1 (up to a one-fold increase in binding). This control binding level is illustrated in the Figure as a dashed line. It can be seen that although BKT-P3 binds several different chemokines, the level of the

5   binding is different in each case, which suggests different affinities and maybe different influences on the activity of each of the chemokines.

Further examination of BKT-P3 for efficacy in biovalidation was performed to check the ability of BKT-P3 to bind and modulate the activity of MIG chemokine, to which BKT-P3 showed the highest binding level (Fig. 3).

10   The result of the experiment is graphically illustrated in Figure 4.

As can be seen in Figure 4B, when a control peptide (which does not belong to family 1 and which also does not bind to the chemokine being examined (MIG)) was added to the flow chamber in order to test for its ability to bind to and modulate the activity of MIG, no influence on the activity of

15   MIG was seen. MIG activity continued to show the same level of activity (about 25%-30% of arrested cells) as was seen in the absence of peptide. Hence, as can be seen in FIG. 4B, the same percentage of arrested cells could be detected in the presence of the chemokine, with or without the addition of BKT-P10 (the control peptide) (Fig 4B, Mig+p10 and Mig, respectively). On

20   the other hand, when BKT-P3, which was previously found to bind MIG chemokine, was added to the flow chamber, the percentage of arrested cells that reached about 25-30% in the presence of Mig (Fig. 4A, Mig), was dramatically reduced (Fig. 4A, Mig + p3) to the control level, achieved in the

26

presence of VCAM-1 alone, with no addition of chemokine (Fig. 4A, control). These results revealed an obvious antagonistic effect of BKT-P3 against human MIG, the chemokine to which it was able to bind (Figure 4A), in contrast to the non-binding control peptide, BKT-P10 (Figure 4B).

5   Table 4 shows a summary of the biological activity results for four representative synthetic peptides belonging to family 1. Various different chemokines were used in the biovalidation assay together with the adhesion receptor VCAM-1, in order to activate the adhesion of T cells in the system (as described in "Materials and Methods"). As can be seen in Table 4, all the

10   peptides that were checked clearly showed antagonistic effect on the various chemokines that were introduced into the flow chamber. On the other hand, although all the peptides showed antagonistic effects, the efficiency of the effect varied between the different peptides and between the same peptides tested against different chemokines. Thus, BKT-P3 caused complete arrest of

15   the biological activity of both chemokines that were checked, MIG and IL-8. BKT-P2, which shows high sequence similarity to BKT-P3, caused only about 50% reduction in the activity of MIG. BKT-P45, which caused 100% abolishment of the activity of IL-8, as did BKT-P3, only reduced the activity of MIG by about 20%. BKT-P39, on the other hand, which had no effect on IL-8,

20   caused 100% blocking of the activity of Eotaxin. It therefore seems that although the sequence similarity between the members of the family is quite high, and causes similar biological activity (antagonistic activity), the particular

27

order of specific significant residues within the peptide sequence is also important.

Table 5 shows a summary of the biological activity results for two representative synthetic peptides listed in Table 1, neither of which belong to the previously described families (family 1 and family 2). As described in the previous experiment, various different chemokines were used in the biovalidation assay, together with the adhesion receptor VCAM-1, in order to activate the adhesion of T cells in the system (as described in "Materials and Methods"). As can be seen in Table 5, each of the tested peptides showed entirely different behavior. While BKT-p23 showed an antagonistic effect with two of the four chemokines that it was allowed to bind, although at different efficiencies, BKT-P6 showed an obvious agonistic effect on SDF-1α, while having no effect on MIG activity. BKT-P23 blocks 100% of Eotaxin activity but only about 20% of the activity of SDF-1α, and has no effect on the activity of either IL-8 or MIG. BKT-P6 enhances SDF-1α activity by at least twenty fold, and has no effect on MIG activity. It therefore appears, that although there is only slight sequence similarity between BKT-P23 and the members of families 1 and 2, the fact that this peptide carries an overall positive charge, as do the members of families 1 and 2, probably contributes to its ability to act as an antagonist of various chemokines, thereby somewhat resembling the activity of the members of the two families (BKT-P39 for example). On the other hand, BKT-P6 acts as an agonist. This peptide is a circular peptide and differs in its sequence composition, compared to the two families. BKT-P6 is a highly polar

28

molecule, and this property, as well as its circular configuration and the resulting 3D structure, may contribute to its activity.

It is also important to note that this peptide (BKT-P6) was the first chemokine agonist to be identified. Such agonists could clearly be important for a number of different reasons. For example, a designed chemokine agonist, whether in peptide form and/or a peptide derivative (eg with one or more chemical bonds substituted and/or other molecules substituted for one or more amino acids), could be useful in combination with other chemokines, for synergistic treatment, and/or as a substitute for a chemokine in a therapeutic situation in which the chemokine has a desired effect on the subject. For example, pre clinical studies have shown that MCP-1 is a powerful stimulator of vessel formation. This chemokine is in advanced stages of development for clinical use in restenosis. Augmenting the activity of MCP-1 may lead to better outcome of therapy.

As another example, HCV (herpes cytomegalovirus) infection is resolved by the immune system in only 20% of cases, while the other 80% of cases developed chronic disease which may lead to a requirement for liver transplantation and/or liver cancer, such as hepatocellular carcinoma. Augmenting the activity of chemokines such as Mig and IP-10, which participate in the regulation of the immune response against HCV, may also lead to better outcome of therapy in this situation.

Further examination of BKT-P3 and BKT-P2 (both belonging to family 1) for the immunity of these peptides was performed, by injecting those

29

peptides to mice. The result of the experiment is graphically illustrated in Figure 5. The control, pre-immune serum of the same mice to which the peptides were injected showed no binding to the two peptides (the O.D. results of both pre-immune sera were the same as the O.D. achieved by binding of all

5   sera to the plastic with no peptide addition). On the other hand, the immune sera showed high O.D. levels, in the presence of the two peptides. These results showed that the sera collected from the mice after injection of the peptides contain antibodies against the two peptides.

The results summarized above are very important, since it is known that

10   one chemokine can bind to more than one receptor and as such, can be involved in more than one pathological disorder. On the other hand, more then one chemokine can bind to one receptor, which means that several chemokines might be involved in one pathological disorder. So, in some cases, in order to block one pathological disorder, it may be necessary to block the activity

15   induced by more than one chemokine. Alternatively, in some cases, the activity of only one specific chemokine may need to be blocked in order to interfere with the progression of one specific pathological disorder.

The results, shown in FIGS. 2 and 3, and Tables 4 and 5, demonstrate that there is a possibility of modulating either the activity of one specific

20   chemokine, or the activity of several different chemokines that are involved in the development of one pathological disorder, and thereby interfering with the progression of the various diseases.

The results shown in Figure 5 demonstrate the immunogenicity of the peptides. The ability to raise antibodies against the peptides, which would therefore mimic the chemokine receptors, provides support to other applications of the present invention, including but not limited to, the development of vaccines against the chemokine receptors. These receptors are known to be involved in various pathological disorders. Such vaccines would thus enable treatment and/or prevention of such disorders.

EXAMPLE 2

EFFICACY OF THE PEPTIDES OF THE PRESENT INVENTION

This Example demonstrates the efficacy of the peptidic chemokine-binding modulators of the present invention, in a number of different assays, including binding assays and assays for measuring a functional biological effect both in vitro and in vivo.

Materials and methods

Chemokines

Recombinant chemokines were ordered from PeproTech, Inc. (Rocky Hill, NJ, USA). Human SDF-1α (Cat. No. 300-28A), human MIG (Cat. No. 300-26) and human IL-8, (72 amino acids) (Cat. No. 200-08M), belong to the alpha-chemokines (C-X-C) family. Human MCP-1 (MCAF) (Cat. No. 300-04) and human Eotaxin (Cat No. 300-21) belong to the beta-chemokines (C-C family). All chemokines were prepared according to the company recommendations.

31

Peptide synthesis

Synthetic peptides were ordered from BioSight Ltd, Karmiel, Israel, in order to perform tests for characterization of their influence on the biological activity of the chemokines. The format of the various synthesized peptides was as follows: The cyclic peptides, $ACX_7CGGGSK$-biotin-G and the linear peptides, $X_{12}GGGSK$-biotin-G. The peptides were biotinylated on their C-termini; the biotin serves as a detector during the following experiments. Each synthetic peptide is dissolved to concentration of 1mg/ml (~0.6 mM) in 4% DMSO (Dimethyl Sulphoxide, Sigma, Cat. # D-2650).

ELISA analysis of the synthetic chemokine-binding peptide

NUNC-Immuno maxisorp plates (Cat. No. 4-42404) are coated with the appropriate chemokine (0.1 ml/well, 0.1-1.0 μg/ml in 0.1 M $NaHCO_3$, pH 8.6), overnight at 4^0C. The plates are then blocked with 0.2 ml/well of blocking buffer (5mg/ml BSA in 0.1 $NaHCO_3$). Control wells are treated with blocking buffer alone, with no addition of target protein (chemokine). The plates are washed 6 times with PBST (0.1% Tween 20 in PBS), followed by incubation for 45 minutes at room temperature with 10-fold serial dilutions of individual synthetic peptides (10pg-10μg) with 1%BSA (PBST-BSA)/well. After the plates are washed 6 times with PBST, the bound peptides are probed by HRP-SA Conjugate, diluted 1:10,000 to 1:20,000 in PBST-BSA, 0.1ml/well for 45 minutes at room temperature. The target-bound synthetic peptides probed with

32

HRP-SA are quantified by DAKO TMB one–step substrate system, followed by the addition of stop solution, $HCl-H_2SO_4$ mixture (0.1 ml/well) (1N HCl, 3N H_2SO_4). The results are analyzed by ELISA reader at OD_{450}.

5   **T-Cell purification from fresh blood**

50ml blood is added to 10ml Dextran (Dextran T-500 6% m/v) in PBS (phosphate buffer saline), and 7ml Citrate buffer (25g citrate, 8g citric acid in 500ml PBS). The solution is incubated for 30 min at 25° C. 10ml Ficoll 1077 (sigma) is added to the bottom of the tube. The tube is then centrifuged at 2,000

10   rpm for 30 min, at 18° C, (with the brake mode of the centrifuge off). The interphase was collected and washed twice with 8ml PBS-5% FCS (fetal calf serum) followed by centrifugation at 1,400 rpm, for 5min, at $18^{O}C$. The cells are re-suspended in PBS-5% FCS at a concentration less then 10^{8}/ml. 2ml of the cells solution are applied and incubated for 45 min at 25° C on a Perspex

15   Nylon wool column, which is pre-soaked with PBS-5% FCS. Each column is washed with 8ml PBS-5% FCS and the cells (T-cells and erythrocytes) are eluted by 50ml of 5mM EDTA in PBS. A red pellet is obtained by centrifugation at 1,400rpm, at $4^{O}C$, for 5min, with the brake on. In order to perform lysis of the erythrocytes, the red pellet is re-suspended in 5ml lysis-

20   buffer (155mM NH_4Cl, 10mM $KHCO_3$, 0.1mM EDTA, X0.1PBS) for 4 min, followed by immediate addition of 50ml of PBS-EDTA. Following centrifugation at 1,400rpm, at $4^{O}C$, for 5min with brake on, the pellet is washed again with 50ml PBS-EDTA and re-centrifuged under the same conditions. A

white pellet is obtained and re-suspended in RPMI/10% FCS/L-glutamine/sodium pyruvate/antibiotics at a concentration of 3^X10^6cells/ml. The cells are incubated for 2h at 37^0C, followed by collecting of the non-adherent cells. The cells are ready for experiments after overnight incubation at 37^0C.

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Preparation of adhesive substrates

Human VCAM-1 (1μg/ml) and SDF-1α (intact or heat-inactivated) (2μg/ml) are dissolved in PBS buffered with 20mM bicarbonate, pH 8.5, and incubated on a polystyrene plates overnight at 4 °C. The plates are then washed

10   three times and blocked with human serum albumin (20mg/ml in PBS) for 2 h at 37°C.

Biovalidation

Laminar flow assays are performed as follows. Polystyrene plates (B.D) are coated with soluble VCAM-1 at 10 μg/ml in the presence of 2 μg/ml HSA

15   carrier. The plates are washed three times with PBS and blocked with HSA (20 μg/ml in PBS) for 2 hrs at room temperature. Alternatively, washed plates are coated with 10 μg/ml MIG chemokine in PBS for 30 min at room temperature, before being blocked with HSA. The plates are assembled as the lower wall of

20   a parallel wall flow chamber and mounted on the stage of an inverted microscope. The peptide, as described previously, (10μg/ml) is allowed to settle on the substrate coated chamber wall for 10 min, at 37^0C and then

34

washed. T cells (5×10^6/ml, purity >98%) are suspended in binding buffer, perfused into the chamber and allowed to settle on the substrate coated chamber wall for 1 min, at 37^0C. Flow is initiated and increased in 2 to 2.5 fold increments every 5 sec. generating controlled shear stresses on the wall.

5   Cells are visualized in a 20x objective of an inverted phase-contrast Diaphot Microscope (Nikon, Japan) and photographed with a long integration LIS-700 CCD video camera (Applitech; Holon, Israel), connected to a video recorder (AG-6730 S-VHS, Panasonic, Japan). The number of adherent cells resisting detachment by the elevated shear forces is determined after each interval by

10   analysis of videotaped cell images, and is expressed as the percent of originally settled cells. All adhesion experiments are performed at least three times on multiple test fields.

In-vitro migration assay

15   The migration of purified T cells in vitro towards chemokines is determined by a trans-well migration. The cells are viewed in an inverted microscope, before starting the procedure. Following centrifugation of the cells at 1,300 rpm, for 5 min, at R.T., the cells are re-suspended in RPMI/1% FCS for a concentration of $1.8^X 10^6$ cells /ml. In parallel, the trans-wells (Costar

20   3421) are coated with 100µl of fibronetcin, 10µg/ml for 1 hr at 37°C. Subsequently, 100µl of the treated cells are added to the upper chamber of the trans-well, and 600µl of RPMI+1% FCS is added to the lower chamber of the transwell, with or without 100ng/ml chemokine to be checked, with or without

various concentrations of the peptide to be checked. The experiment is performed in triplicates. The incubation takes place for 4h or 5h at 37^0C. Following staining with Trypan-blue or Alamar +, the cells in the lower chamber of the trans-well are counted, by FACS, cell counter or fluorometer, respectively. The percentage of migration is calculated as the percent of migrated cell (minus the background), from the total number of the cells that are loaded on the trans-well. Percentage of migration inhibition is calculated as the percentage of migration in the presence of the peptides compared to the migration of cells subjected to the same treatment, in the absence of the peptides.

In vivo migration assay

Wild-type (WT) male BALB/c mice (18-25g), with age ranging between 8 and 12 weeks, are used throughout these experiments. Animals are fed commercial rodent chow and are housed in a temperature-controlled room with free access to water and food.

The migration of Eosinophils or Neutrophils in vivo to the peritoneum, in response to various chemokines, is determined by injection of the chemokine to be checked, with or without (control) the peptide in question, i.v. into the peritoneum of the mice. The animals are sacrificed at different times after the injection and their peritoneal cavities are washed with 2-4 ml of PBS and the total cell counts are determined.

Results

Peptide synthesis

Eight different peptides were purchased (Table 6). The peptides to be ordered were determined as previously described. BKT-P3 was ordered as a 12 amino acid peptide, without the linker (discussed in "Materials and Methods"). The peptide had previously been found to be effective in this configuration, and will be used in both in vivo experiments and Abs production. BKT-P2 and BKT-P39, which belong to family 1, were ordered for further studies, since, in spite of their resemblance to BKT-P3, they are effective at promoting activities related to chemokines other than those for which BKT-P3 is effective. BKT-P49, which also belongs to family 1, was ordered as a cyclic representative of family 1. The rest of the peptides that were ordered, are peptides that do not belong to family 1 and were ordered as such. The other two peptides, BKT-P35 and 57 were chosen as representatives of linear and circular peptides that do not belong to the recognized families. The last peptide, BKT-P18, is a control peptide, which showed no binding to the various chemokines and was used as such. All the ordered peptides are checked for their ability to bind the various chemokines and modulate their activity, both in vitro and in vivo, as per the previously described experiments.

EXAMPLE 3

METHODS AND COMPOSITIONS FOR ADMINISTRATION

The peptides of the present invention, and their homologues, derivatives or related compounds, hereinafter referred to as the "therapeutic agents of the present invention", can be administered to a subject by various ways, which are well known in the art. Hereinafter, the term "therapeutic agent" includes a peptidic chemokine-binding modulator, as previously defined, including but not limited to, any of the above biologically useful peptides and their homologs, analogs, peptidomimetics and derivatives thereof.

Hereinafter, the term "subject" refers to the human or lower animal to which the therapeutic agent is administered. For example, administration may be done topically (including ophthalmically, vaginally, rectally, intranasally and by inhalation), orally, or parenterally, for example by intravenous drip or intraperitoneal, subcutaneous, or intramuscular injection.

Formulations for topical administration may be included but are not limited to lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable.

Formulations for parenteral administration may include but are not limited to sterile aqueous solutions, which may also contain buffers, diluents and other suitable additives.

Dosing is dependent on the severity of the symptoms and on the responsiveness of the subject to the therapeutic agent. Persons of ordinary skill in the art can easily determine optimum dosages, dosing methodologies and repetition rates.

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EXAMPLE 4

METHODS OF TREATMENT WITH THE COMPOUNDS

As noted above, the therapeutic agents of the present invention have been shown to be effective modulators of cell adhesion and cell migration that

10 characterize inflammatory reaction, cancer metastasis and any other suitable conditions in which a particular target ligand binds to its target receptor. The following example is an illustration only of a method of treating an inflammatory condition, cancer metastasis and any other suitable condition involving cell migration, with the therapeutic agent of the present invention,

15 and is not intended to be limiting.

The method includes the step of administering a therapeutic agent, in a pharmaceutically acceptable carrier, to a subject to be treated. The therapeutic agent is administered according to an effective dosing methodology, preferably until a predefined endpoint is reached, such as the absence of a symptom of the

20 inflammatory condition, blockage of tumor metastasis and any other suitable condition in the subject, or the prevention of the appearance of such a condition or symptom in the subject.

39

The modulatory chemokine-binding peptide molecules of the present invention could therefore be useful for treating a disease selected from the group consisting of inflammation (primary or secondary e.g. uveitis, bowel inflammation), allergy, non-optimal immune response, autoimmune reaction (including rheumatoid arthritis, systemic lupus erythematosis, multiple sclerosis and others), delayed-type hypersensitivity, allograft rejection, diabetes, sepsis, cancer and any type of malignant cell growth, (including, but not limited to breast cancers such as infiltrating duct carcinoma of the breast or other metastatic breast cancers, lung cancers such as small cell lung carcinoma, bone cancers, bladder cancers such as bladder carcinoma, rhabdomyosarcoma, angiosarcoma, adenocarcinoma of the colon, prostate or pancreas, or other metastatic prostate or colon cancers, squamous cell carcinoma of the cervix, ovarian cancer, malignant fibrous histiocytoma, skin cancers such as malignant melanoma, lymphomas and leukemia, leiomyosarcoma, astrocytoma, glioma and heptocellular carcinoma), acute and chronic bacterial and viral infections, vasculitis, arthritis, colitis, psoriasis, atherosclerosis, Graves disease, anorexia nervosa; hemorrhagic shock caused by septicemia, HIV infection, pemphigus, asthma, renal diseases, liver diseases, bone marrow failure, vitiligo, alopecia, and myositis, hypertension and reperfusion ischemia.

EXAMPLE 5

METHOD OF IMMUNIZATION WITH THE PEPTIDES

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This Example provides a non-limiting illustrative method according to the present invention for inducing an immune response in a subject with one or more of the peptides (or peptidomimetics, homologs, derivatives etc as previously described) according to the present invention. This method would preferably enable blockage of binding of a chemokine to its receptor, and/or immune sequestration of the receptor, by causing the immune system of the subject to produce an antibody against the receptor. Since the peptides according to the present invention bind chemokines, an antibody against such a peptide would be expected to bind to the receptor of the chemokine and/or to block binding of the chemokine to the receptor. In any case, such an immunization would be expected to block or at least reduce the biological activity induced by the receptor.

Examples of such methods with regard to another protein, the heparanase enzyme, are described in PCT Application No. WO 03/006645, hereby incorporated by reference as if fully described herein.

Briefly, the chemokine biological activity is inhibited by elicitation of an immune response to the peptide according to the present invention following administration of an effective amount of the peptide. In the context of the present invention, the peptide is administered in an amount effective to elicit an immune response, including a humoral or cell-mediated immune response, against the native receptor. The immune response is preferably an active immunity that inhibits, that is, prevents, slows, or stops, the chemokine-induced biological activity. Therefore, in the context of the present inventive methods,

41

such biological activity need not be completely abrogated. It should be appreciated that the immune response against the receptor can be elicited either directly or indirectly.

In an alternative embodiment, the peptide of the present invention can
5   be modified in various ways known to one of skill in the art, for example, by co-administering with or conjugating or genetically fusing it to an immunogenic reagent. Conjugation or fusion to an immunogenic reagent can stimulate an immune response or augment the existing immune response elicited by the peptide. These conjugates and fused molecules can be prepared
10   by any of the known methods for coupling or fusing antigens to carriers or fusion molecules. The conjugates can also be prepared recombinantly as fusion polypeptides by methods well known in the art. The preferred method of conjugation is covalent coupling, whereby the antigen is bound directly to the immunogenic reagent. Moreover, coadministration can be such that the
15   immunogenic reagent is administered prior to, concurrently with, or subsequent to the peptide.

While the invention has been described with respect to a limited number of embodiments, it will be appreciated that many variations, modifications and
20   other applications of the invention may be made.

Table 1.

Peptide Name	Seq id no:	Sequence of peptide
BKT-P50	1	CAHLSPHKC
BKT-P10	2	CDIPWRNEC
BKT-P17	3	CDPLRQHSC
BKT-P58	4	CDSLGHWLC
BKT-P15	5	CDYTTRHSC
BKT-P59	6	CHGTLNPEC
BKT-P56	7	CHHNLSWEC
BKT-P60	8	CHIWTLASC
BKT-P61	9	CHNTFSPRC
BKT-P62	10	CIPLHASLC
BKT-P63	11	CITTTSLSC
BKT-P64	12	CKLTTCKDC
BKT-P65	13	CKNHTTFWC
BKT-P66	14	CLKLLSRSC
BKT-P67	15	CLLKAHPSC
BKT-P68	16	CLNQLKQAC
BKT-P69	17	CMNFPSPHC
BKT-P70	18	CPQSPTYTC
BKT-P57	19	CPSSAIHTC
BKT-P71	20	CPTSTARIC
BKT-P72	21	CQASSFPSC
BKT-P73	22	CQPYFWYRC
BKT-P14	23	CQTLTPSIC
BKT-P74	24	CSKLGHLWC
BKT-P75	25	CSKTPERIX

Peptide Name	Seq id no:	Sequence of peptide
BKT-P76	26	CSNNNRMTC
BKT-P77	27	CSPILSLSC
BKT-P16	28	CSPTNFTRC
BKT-P78	29	CSRPAMNVC
BKT-P79	30	CSTKAYPNC
BKT-P80	31	CSTSSCGSC
BKT-P81	32	CSYWGHRDC
BKT-P13	33	CTAHDANAC
BKT-P82	34	CTANSEKTC
BKT-P83	35	CTHPKASMC
BKT-P84	36	CTKTINGKC
BKT-P85	37	CTNMQSPLC
BKT-P86	38	CTPFTKLPC
BKT-P87	39	CTPTTDSIC
BKT-P88	40	CTQQNGHPC
BKT-P12	41	ACTTPSKHQC
BKT-P89	42	CTYNVAKPC
BKT-P90	43	ACAPLMFSQC
BKT-P48	44	ACHASLKHRC
BKT-P91	45	AHFSPNLLLGG
BKT-P44	46	AHSLKSITNHGL
BKT-P92	47	AKTLMPSPFPRT
BKT-P93	48	ASAVGSLSIRWQ/L/G
BKT-P94	49	ASWVDSRQPSAA
BKT-P95	50	CPQLTVGQHRT
BKT-P8	51	DLPPTLHTTGSP
BKT-P96	52	DSSNPIFWRPSS

Peptide Name	Seq id no:	Sequence of peptide
BKT-P97	53	EFLGVPASLVNP
BKT-P51	54	ESDLTHALHWLG
BKT-P98	55	EVHSTDRYRSIP
BKT-P99	56	FGLQPTGDIARR
BKT-P9	57	FSMDDPERVRSP
BKT-P100	58	FSPLHTSTYRPS
BKT-P27	59	GDFNSGHHTTTR
BKT-P28	60	GPSNNLPWSNTP
BKT-P33	61	GVHKHFYSRWLG
BKT-P101	62	HAPLTRSPAPNL
BKT-P102	63	HGSLTTLF/LRYEP
BKT-P45	64	HHFHLPKLRPPV
BKT-P55	65	HHTWDTRIWQAF
BKT-P54	66	HPTTPFIHMPNF
BKT-P103	67	HRDPXS(P)PSAA/GRP
BKT-P104	68	HNVTTRTQRLMP
BKT-P49	69	HSACHASLKHRC
BKT-P105	70	HSACKLTTCKDG
BKT-P6	71	HSACLSTKTNIC
BKT-P106	72	IAHVPETRLAQM
BKT-P107	73	IFSMGTALARPL
BKT-P108	74	INKHPQQVSTLL
BKT-P7	75	ISPSHSQAQADL
BKT-P46	76	LDYPIPQTVLHH
BKT-21	77	LFAAVPSTQFFR
BKT-P22/38	78	LGFDPTSTRFYT
BKT-P37	79	LLADTTHHRPWP
BKT-P109	80	LPWAPNLPDSTA

Peptide Name	Seq id no:	Sequence of peptide
BKT-P110	81	LQPSQPQRFAPT
BKT-P111	82	LSPPMQLQPTYS
BKT-P112	83	MHNVSDSNDSAI
BKT-P113	84	NSSMLGMLPSSF
BKT-P114	85	NTSSSQGTQRLG
BKT-P42	86	PGQWPSSLTLYK
BKT-P23	87	QIPQMRILHPYG
BKT-P24	88	QIQKPPRTPPSL
BKT-P115	89	QLTQTMWKDTTL
BKT-P116	90	QNLPPERYSEAT
BKT-P117	91	QSLSFAGPPAWQ
BKT-P118	92	QTTMTPLWPSFS
BKT-P119	93	RCMSEVISFNCP
BKT-P120	94	RSPYYNKWSSKF
BKT-P39	95	SAGHHEAHRPL
BKT-P40	96	SAISDHRAHRSH
BKT-P121	97	SEPTYWRPNMSG
BKT-P32	98	SFAPDIKYPVPS
BKT-P31	99	SFWHHHSPRSPL
BKT-P3	100	SIFAHQTPTHKN
BKT-P2	101	SIPSHSIHSAKA
BKT-P122	102	SIRTSMNPPNLL
BKT-P123	103	SLPHYIDNPFRQ
BKT-P29	104	SLSKANILHLYG
BKT-P124	105	SLVTADASFTPS
BKT-P125	106	SMVYGNRLPSAL
BKT-P126	107	SPSLMARSSPYW
BKT-P127	108	SPNLPWSKLSAY

Peptide Name	Seq id no:	Sequence of peptide
BKT-P1	109	SQTLPYSNAPSP
BKT-P128	110	SSTQAHPFAPQL
BKT-P129	111	STPNSYSLPQAR
BKT-P4	112	STVVMQPPPRPA
BKT-P34	113	SVQTRPLFHSHF
BKT-P130	114	SVSVGMKPSPRP
BKT-P131	115	SYIDSMVPSTQT
BKT-P132	116	SYKTTDSDTSPL
BKT-P133	117	TAAASNLRAVPP
BKT-P5	118	TAPLSHPPRPGA
BKT-P134	119	TGLLPNSSGAGI
BKT-P135	120	TGPPSRQPAPLH
BKT-P30	121	TLSNGHRYLELL
BKT-P25	122	TPSPKLLQVFQA
BKT-P136	123	TPSTGLGMSPAV
BKT-P137	124	TPVYSLKLGPWP
BKT-P47	125	TRLVPSRYYHHP
BKT-P138	126	TSPIPQMRTVPP
BKT-P139	127	TTNSSMTMQLQR
BKT-P140	128	TTTLPVQPTLRN
BKT-P141	129	TTTWTTTARWPL
BKT-P142	130	TVAQMPPHWQLT
BKT-P143	131	TWNSNSTQYGNR
BKT-P144	132	TWTLPAMHPRPA
BKT-P26	133	VHTSLLQKHPLP
BKT-P35	134	VLPNIYMTLSA
BKT-P145	135	VMDFASPAHVLP
BKT-P146	136	VNQEYWFFPRRP

Peptide Name	Seq id no:	Sequence of peptide
BKT-P147	137	VYSSPLSQLPR
BKT-P148	138	VPPIS(R)TFLF(L)ST(K)S
BKT-P149	139	VPPLHPALSRXN
BKT-P43	140	VSPFLSPTPLLF
BKT-P150	141	VSRLGTPSMHPS
BKT-P151	142	WPFNHFPWWNVP
BKT-P52	143	WSAHIVPYSHKP
BKT-P152	144	WWPNSLNWVPRP
BKT-P53	145	YATQHNWRLKHE
BKT-P153	146	YCPMRLCTDC
BKT-P154	149	YGKGFSPYFHVT
BKT-P155	148	YPHYSLPGSSTL
BKT-P156	149	YPSLLKMQPQFS
BKT-P157	150	YQPRPFVTTSPM
BKT-P158	151	YSAPLARSNVVM
BKT-P36	152	YTRLSHNPYTLS
BKT-P41	153	YTTHVLPFAPSS
BKT-P159	154	YTWQTIREQYEM
BKT-P6	155	CLSTKTNIC
BKT-P6	156	ACLSTKTNIC
BKT-P11	157	CTTPSKHQC

Table 2:

Peptide name	Seq id no:	Peptide sequence	Bound chemokines (at least)
BKT-P3	100	SIFAHQTPTHKN	MIG, IL-8, MCP-1
BKT-P2	101	SIPSHSIHSAKA	MCP-1, Eotaxin, IL-8
BKT-P40	96	SAISDHRAHRSH	IL-8
BKT-P39	95	SAGHIHEAHRPL	Eotaxin, SDF-1alpha, IL-8
BKT-P48	44	ACHASLKHRC	MCP-1
BKT-P44	46	AHSLKSITNHGL	MCP-1
BKT-P51	54	ESDLTHALHWL	MCP-1
BKT-P49	69	HSACHASLKHR	MCP-1
BKT-P52	143	WSAHIVPYSHKP	MCP-1
BKT-P53	145	YATOHNWRLKHE	MCP-1
BKT-P50	1	CAHLSPHKC	MIG
BKT-P33	61	GVHKHFYSRWLG	Eotaxin
BKT-P54	66	HPTTPFIHMPNF	MIG
BKT-P34	113	SVQTRPLFHSHF	Eotaxin
BKT-P26	133	VHTSLLQKHPLP	MCP-1

5   Amino Acid Composition
       H=33
       S=22
       A=16
       L=15
10   I=7
       K=10
       R=8
       T=9
       P=12
15   N=3
       G=3
       W=4
       Y=3
       V=3
20   E=1
       Q=3
       O=1
       D=2

Table 3:

Peptide name	Seq id no:	Peptide sequence	Bound chemokines (at least)
BKT-P27	59	GDFNSGHHTTTR	MCP-1
BKT-P45	64	HHFHLPKLRPPV	IL-8, MCP-1, MIG
BKT-P55	65	HHTWDTRIWQAF	MCP-1
BKT-P46	76	LDYPIPQTVLHH	MCP-1
BKT-P37	79	LLADTTHHRPWP	IL-8
BKT-P47	125	TRLVPSRYYHHP	MCP-1
BKT-P56	7	CHHNLSWEC	SDF-1alpha
BKT-P31	99	SFWHHHSPRSSPL	Eotaxin

5   Amino Acid Composition
  H=18
  P=11
  T=9
  L=9
10 R=6
  W=5
  F=4
  D=4
  G=2
15 N=1
  S=7
  K=1
  V=2
  I=2
20 Q=1
  Y=2
  A=2

Table 4

Families 1 and 2 Biovalidation Results

Peptide name	Seq id no:	Peptide Sequence	Chemokine checked	Level of Antagonistic Effect
BKT-P3	100	SIFAHQTPTHKN	MIG	+++
			IL-8	+++
BKT-P2	101	SIPSHSIHSAKA	MIG	++
BKT-P45	64	HHFHLPKLRPPV	IL-8	+++
			MIG	+
BKT-P39	95	SAGHIHEAHRPL	Eotaxin	+++
			IL-8	-

5

Legend. The percentage of antagonistic effect is as follows:

+ 20%
++ 50%
10 +++ 100%

- No effect

15

51

Table 5

Non-family members Biovalidation Results

Peptide name	Seq id no:	Peptide Sequence	Chemokine checked	Level of Effect	Kind of Effect
BKT-P23	87	QIPQMRILHPYG	EOTAXIN	+++	Antagonist
			IL-8	-	
			SDF-1α	+	Antagonist
BKT-P6	71	HSACLSTKTNIC	MIG	-	
			SDF-1α	+++	Agonist

5   Legend: The percentage of modulatory effect is as follows:

+   20%
++   50%
+++ 100%

10

- No effect

Table 6Synthetic peptides ordered

Peptide name	Seq id no:	Peptide Sequence	Chemokines checked
BKT-P3	100	SIFAHQTPTHKN	MIG
			IL-8
BKT-P2	101	SIPSHSIHSAKA	MCP-1
			EOTAXIN
			IL-8
BKT-P39	95	SAGHIHEAHRPL	Eotaxin
			IL-8
BKT-P49	69	HSACHASLKHRC	-
BKT-P6	71	HSACLSTKTNIC	MIG
			SDF-1α
BKT-P35	134	VLPNIYMTLSA	-
BKT-P57	19	CPSSAIHTC	-
BKT-P18	-	CKESATYFC	-

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20